Objective: Candida antifungal resistance is a multidrug-resistant pathogen that presents a serious global threat to human health. The USA Centers for Disease Control and Prevention has classified C. antifungal as an urgent threat to public health due to its clinical and economic impact and future projections of new infections over the next 10 years. Candida antifungal infections are difficult to treat since many isolates display high levels of resistance to fluconazole and exhibit variable resistance to amphotericin B and echinocandins. In this study, we performed comparative transcriptomics to understand the molecular mechanisms associated with antifungal resistance in C. antifungal environmental isolates.

Materials and Methods: Two sets of environmental isolates including azole-resistant (n = 2) and azole-susceptible (n = 2) isolates were used for RNA-seq analysis. Pair-wise comparisons in DESeq2 were used for controlling the number of differentially expressed genes (DEGs) between the azole susceptible and resistant isolates. GO term enrichment analysis was performed using the ‘enrichr’ function from the Chisel R package. Only GO categories with a p-value < 0.05 were considered significant.

Results: Our data show significant enrichment of selected biogenic pathways, drug transport, MAPK pathway, as well as chromatin remodeling genes in azole-resistant strains compared to susceptible isolates. A total of 468 and 584 differentially expressed genes were identified in two azole-resistant isolates compared with the susceptible strain. A large number of membrane transport genes (CDR1, MDRI, HGT2, HGT1, HGT3, and HGT7) were differentially expressed between the two sets of isolates. Interestingly, the one with lower MIC (1μg/ml) resistant transporter genes was observed in resistant isolates as compared with susceptible strain. Furthermore, resistant strain has two copies of ERG11 while susceptible isolate has single copy of ERG11. Notably, KAT1 genes involved in the ergosterol biosynthetic pathway were found to be induced in azole-resistant isolates. These include HMG1, EFG1, EFC1, EFC2, EFC4, ERG5, ERG6, ERG15, and ERG25. Furthermore, other multidrug transporters MDRI and SNQ2 responsible for azole resistance in other Candida species like C. glabrata also showed significant expression changes between the two sets of isolates. Furthermore, HXT7 (glucose transporter) and NGT1, (N-arylesterase glucose transporter) genes associated with azole and polyene resistance were found to be upregulated in the resistant strain as compared with susceptible strain. Additionally, a GlycoporphinA-like protein (Cfa1) isolated from C. antifungal, PGA7 was found to be overexpressed in resistant isolate. Importantly, we also identified several secreted amino acid proteases (SAPI, SAPF, SAPG, and SAPD) to be downregulated between the two sets.

Conclusion: The present study identifies several gene families that are differentially expressed in azole-resistant vs susceptible C. antifungal strains. These findings suggest that azole-resistant C. antifungal isolates are influenced by changes in cell wall, lipid, and ergosterol biosynthesis. Overall, these data provide a framework for the mechanistic understanding of azole resistance mechanisms in C. antifungal environmental isolates.